FISH YIELDS AND ECONOMIC BENEFITS OF TILAPIA/*CLARIAS* POLYCULTURE IN FERTILIZED PONDS RECEIVING COMMERCIAL FEEDS OR PELLETED AGRICULTURAL BY-PRODUCTS

Ninth Work Plan, Feeds and Fertilizers Research 2A (9FFR2A)
Final Report

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ABSTRACT

Stable carbon and nitrogen isotopes were used to obtain estimates of the contribution of natural and supplemental feeds to the nutrition of *Oreochromis niloticus* in ponds (free-swimming or caged) receiving different inputs in Sagana, Kenya. Three dietary treatments were employed in the pond study: 1) the test diet; 2) a pig finisher diet; and 3) a rice bran diet. Feeding rates and fertilization regimes are detailed in the report for 9FFR2. For isotope analysis, samples of *Oreochromis* (free-swimming and caged) and plankton were taken from ponds in Sagana three times (January, March, and May) during the study. The carbon and nitrogen isotope ratios of the diets were analyzed once. Modest fish growth during the study on all dietary treatments (the fish acquired ≤ 50% of their final weight between January and May) limited the application of the stable isotope technique for determining the relative assimilation of plankton and the different diets. The patterns of change in the $\delta^{13}C$ and $\delta^{15}N$ of free-swimming and caged *Oreochromis* and plankton over time and their possible interpretation were described within and between treatments.

INTRODUCTION

Stable carbon and nitrogen isotope analyses are useful techniques to obtain quantitative estimates of the relative contributions of different food sources to the nutrition of aquatic animals in ponds (Schroeder, 1983; Anderson et al., 1987; Yoshioka et al., 1989; Lochmann and Phillips, 1996; Lochmann et al., 2001). Presumably, isotope ratios of the fish will resemble those of the food(s) they assimilate most. The carbon isotopes are used to delineate the directions of carbon flow throughout a food web, and the nitrogen isotopes can be used to indicate the trophic level of different organisms in a pond. The double tracer technique is usually more powerful than the use of a single isotope in determining patterns of nutrient flow in aquatic systems, particularly when organisms of multiple trophic levels are present. In this study stable carbon and nitrogen isotope ratios of diets and plankton were compared to those of *Oreochromis* (free-swimming in ponds or confined to cages in the same ponds). Although *Clarias* was included in the study as a predator, insufficient data were collected on this species to determine its nutrient sources during the study. The caged *Oreochromis* did not receive any prepared feed and were expected to consume mostly plankton, while the free-swimming fish in the same pond had access to both an artificial input (one of the three diets) and plankton. A comparison of the stable carbon and nitrogen isotope ratios ($\delta^{13}C$ and $\delta^{15}N$, respectively) between the two groups was expected to reveal the additional benefits, if any, of adding supplemental inputs (diets) to ponds containing plankton. We anticipated using the results to optimize nutrient utilization and minimize feed and fertilizer costs for *Oreochromis* production in Kenya and possibly other locations.

METHODS AND MATERIALS

Pond production data such as stocking rates, feeding rates, and fertilization regimes are detailed in the final report of Liti et al. (see "Growth performance and economic benefits of *Oreochromis niloticus*/*Clarias gariepinus* polyculture fed on three supplementary feeds in fertilized tropical ponds," 9FFR2; pp. 11–16 of this report) and will not be repeated here. The three diets tested in this study were a test diet, a pig finishing ration, and rice bran. Each diet was fed to fish in four ponds, for a total of twelve ponds, for approximately seven months. Because some of the experimental samples were lost in Sagana prior to analysis, this report includes data for only five months of the study (January through May). Dry matter and ash analysis of the diets was conducted at the University of Arkansas at Pine Bluff using standard methods (Association of Official Analytical Chemists, 1984); protein analysis was by the Kjeldahl method, and lipids were analyzed using a Folch extraction (Folch et al., 1957).

Plankton was the main natural component of the pond system assumed to contribute to the nutritional status of *Oreochromis* and *Clarias*. Therefore, samples of the plankton and the fish (*Oreochromis*, both free-swimming and caged) collected in January, March, and May were subjected to carbon and nitrogen isotope ratio analyses. There were not sufficient numbers of *Clarias* to sample more than once, so isotope data for *Clarias* were included for qualitative comparison only. Plankton was treated two different ways at the March sampling period only. Some of the plankton (called Plankton in the Results section) was collected by net and not treated further, like at the other sampling periods. The rest of the plankton collected by net was centrifuged to concentrate the phytoplankton. This phytoplankton concentrate was called Plankton 2 in the Results. The isotope values of both samples were analyzed. Diets, fish, and plankton were prepared for stable isotope analysis as described previously (Lochmann and Phillips, 1996). The analyses were conducted at the Stable Isotope Laboratory at the University of Arkansas, Fayetteville, Arkansas, using a micromass isotope ratio mass spectrometer.

RESULTS AND DISCUSSION

Proximate composition of the diets is shown in Table 1. The test diet and the pig finisher diet were similar to each other in proximate composition, and the rice bran was lower in protein and higher in ash than the other diets. Several batches of each diet were formulated during the study, and rice bran varied the most in proximate composition between batches.

The test diet and the pig finisher diet have similar $\delta^{13}C$ and $\delta^{15}N$ values (Tables 2 and 3). Rice bran had much more negative $\delta^{13}C$ values and positive $\delta^{15}N$ values than the other two diets (Tables 2 and 3). *Oreochromis* and plankton samples collected 5 January 2000 were used as the initial samples for isotope data. By January there were already significant differences in the $\delta^{13}C$ values of the free-swimming fish and plankton in ponds receiving rice bran (Table 2) and in the $\delta^{15}N$ values of the plankton in ponds receiving rice bran (Table 3). By contrast, there were no differences in $\delta^{15}N$ of free-swimming or caged tilapia in ponds receiving any of the diets in January. There were also no differences in $\delta^{13}C$ of caged tilapia in ponds receiving any of the diets in January (Table 2), probably reflecting their lower rate of weight gain and thus assimilation of different nutrients, as reflected by their isotope values.

By March the $\delta^{13}C$ values of the free-swimming and caged tilapia as well as the plankton were all significantly more positive in ponds receiving the test diet or the pig finisher diet compared to ponds receiving rice bran (Table 2). This trend continued in the fish and plankton samples taken in May (Table 2). The $\delta^{15}N$ values of free-swimming tilapia, by contrast, were distinctly different among all three diets (Table 3), while there were no significant differences in $\delta^{15}N$ of caged tilapia or plankton among diets in March (Table 3). This trend in the $\delta^{15}N$ values continued into May (Table 3).

Figure 1 shows the change in $\delta^{13}C$ values in ponds receiving each of the diets over time. The test diet (Figure 1a) was isotopically distinct from the plankton (both Plankton and Plankton 2) for the whole period. The values for plankton and both free-swimming (Pond) and caged fish stayed within a fairly narrow range throughout the study, and there was no large decrease in $\delta^{13}C$ of the fish, which would have indicated a high rate of assimilation of the test diet. However, free-swimming fish gained only about 50% of their final body weight during the period of observation. Generally, a weight increase of 250% is needed to determine whether a diet will be assimilated to a great degree (Anderson et al., 1987). The pattern of change in $\delta^{13}C$ over time was similar in ponds that received the pig finisher diet (Figure 1a). In this case the isotope values of the free-swimming tilapia were consistently more positive than those of the caged tilapia, indicating a higher rate of assimilation of the pig finisher compared to the

Table 1. Proximate composition (%) of diets used in a feeding trial with free-swimming and caged tilapia in ponds in Sagana, Kenya.[1]

Diet	Protein[2]	Lipid[3]	Dry Matter[4]	Ash[5]
Test	11.52 ± 0.00	6.33 ± 0.04	87.80 ± 0.00	8.10 ± 0.07
Pig Finisher	11.59 ± 0.49	4.18 ± 0.32	87.82 ± 0.00	9.04 ± 1.16
Rice Bran	8.10 ± 0.61	4.12 ± 0.18	90.59 ± 0.00	19.41 ± 0.08

1 Values are means of two replicates ± SD for the first batch of diets used in the experiment.
2 Protein in subsequent batches of the test diet was within 1% of the first batch. Protein ranged from 9.8 to 12.4% for subsequent batches of the pig finisher and 4.7 to 8.1% for subsequent batches of rice bran.
3 Lipid in subsequent batches of all diets was within 1% of the values for batch 1.
4 Dry matter in subsequent batches of all diets was within 2% of the values for batch 1.
5 Ash in subsequent batches of pig finisher was within 1% of the value for batch 1. Ash in subsequent batches of rice bran ranged from 15.5 to 21.1%.

Table 2. Stable carbon isotope ratios ($\delta^{13}C$ in ‰) of free-swimming tilapia in ponds, tilapia in cages in the same ponds, and plankton sampled in January, March, and May 2000 in Sagana, Kenya.[1] The $\delta^{13}C$ of the diets is indicated in parentheses.

Diet	Free-Swimming Tilapia	Caged Tilapia	Plankton
JANUARY			
Test (–16.9)	–18.8 ± 0.5 [a]	–19.4 ± 0.5	–20.5 ± 2.0 [a]
Pig Finisher (–16.2)	–18.9 ± 0.8 [a]	–19.6 ± 0.8	–21.2 ± 1.1 [a]
Rice Bran (–27.6)	–20.5 ± 0.4 [b]	–19.8 ± 0.2	–23.7 ± 1.2 [b]
MARCH			
Test (–16.9)	–18.5 ± 0.6 [a]	–18.8 ± 0.8 [a]	–18.1 ± 1.2 [a]
Pig Finisher (–16.2)	–18.3 ± 0.3 [a]	–18.9 ± 0.5 [a]	–18.9 ± 1.6 [a]
Rice Bran (–27.6)	–21.2 ± 2.3 [b]	–20.5 ± 0.4 [b]	–22.2 ± 1.0 [b]
MAY[2]			
Test (–16.9)	–18.0 ± 0.4 [a]	–17.7 ± 0.9 [a]	–18.5 ± 0.6 [a]
Pig Finisher (–16.2)	–18.0 ± 0.2 [a]	–18.7 ± 0.6 [a]	–18.2 ± 1.1 [a]
Rice Bran (–27.6)	–22.0 ± 1.0 [b]	–20.7 ± 0.4 [b]	–23.1 ± 0.5 [b]

1 Values are means of four replicates ± SD.
2 *Clarias* (stocked to control tilapia reproduction) samples were available for isotope analysis only in May. The mean $\delta^{13}C$ values for *Clarias* fed the test diet, pig finisher, and rice bran were –17.0 ± 0.5, –17.7 ± 0.8, and –0.8 ± 0.6‰, respectively. Fish fed the rice bran had significantly more negative $\delta^{13}C$ than those fed the test diet or pig finisher.
a,b Means for each month in columns with different letters are significantly different ($P < 0.05$) according to Fisher's Least Significant Difference test.

Table 3. Stable nitrogen isotope ratios ($\delta^{15}N$ in ‰) of free-swimming tilapia in ponds, tilapia in cages in the same ponds, and plankton sampled in January, March, and May 2000 in Sagana, Kenya.[1] The $\delta^{15}N$ of the diets is indicated in parentheses.

Diet	Free-Swimming Tilapia	Caged Tilapia	Plankton
JANUARY			
Test (3.8)	7.9 ± 0.5	8.1 ± 0.1	5.4 ± 0.7 [a]
Pig Finisher (3.9)	8.0 ± 0.5	8.2 ± 0.3	3.8 ± 1.0 [b]
Rice Bran (6.8)	8.2 ± 0.5	8.2 ± 0.6	5.3 ± 0.6 [a]
MARCH			
Test (3.8)	6.9 ± 0.5 [a]	7.9 ± 0.5	4.2 ± 0.4
Pig Finisher (3.9)	7.3 ± 0.8 [b]	7.6 ± 0.6	4.0 ± 0.7
Rice Bran (6.8)	8.2 ± 0.3 [c]	8.0 ± 0.4	4.2 ± 0.7
MAY[2]			
Test (3.8)	7.2 ± 0.4 [ab]	7.4 ± 1.2	2.9 ± 1.3
Pig Finisher (3.9)	6.9 ± 0.9 [a]	7.5 ± 0.5	3.4 ± 0.6
Rice Bran (6.8)	8.1 ± 0.3 [b]	7.9 ± 0.4	3.0 ± 4.5

1 Values are means of four replicates ± SD.
2 *Clarias* (stocked to control tilapia reproduction) samples were available for isotope analysis only.
a,b,c Means for each month in columns with different letters are significantly different ($P < 0.05$) according to Fisher's Least Significant Difference test.

caged tilapia. The $\delta^{13}C$ value of the plankton in the ponds receiving the pig finisher steadily increased (became more positive), possibly reflecting assimilation of the diet as well. The pattern of $\delta^{13}C$ values in ponds that received the rice bran was distinct from that of ponds that received the other two diets (Figure 1c). The rice bran was isotopically distinct from the plankton in this case also, but the diet value was much more negative than all other components in the pond, whereas the other two diets were much more positive than the other pond components (Figures 1a and b). The $\delta^{13}C$ of both free-swimming and caged tilapia gradually became more negative during the study. However, in this case the $\delta^{13}C$ of both the diet and the plankton were more negative than the fish (free-swimming and caged) initially. Therefore, it is not possible to determine the relative contributions of the plankton and the diet to the final $\delta^{13}C$. The $\delta^{13}C$ of the plankton in this treatment first increased then decreased during the study. The reasons for this pattern were not clear.

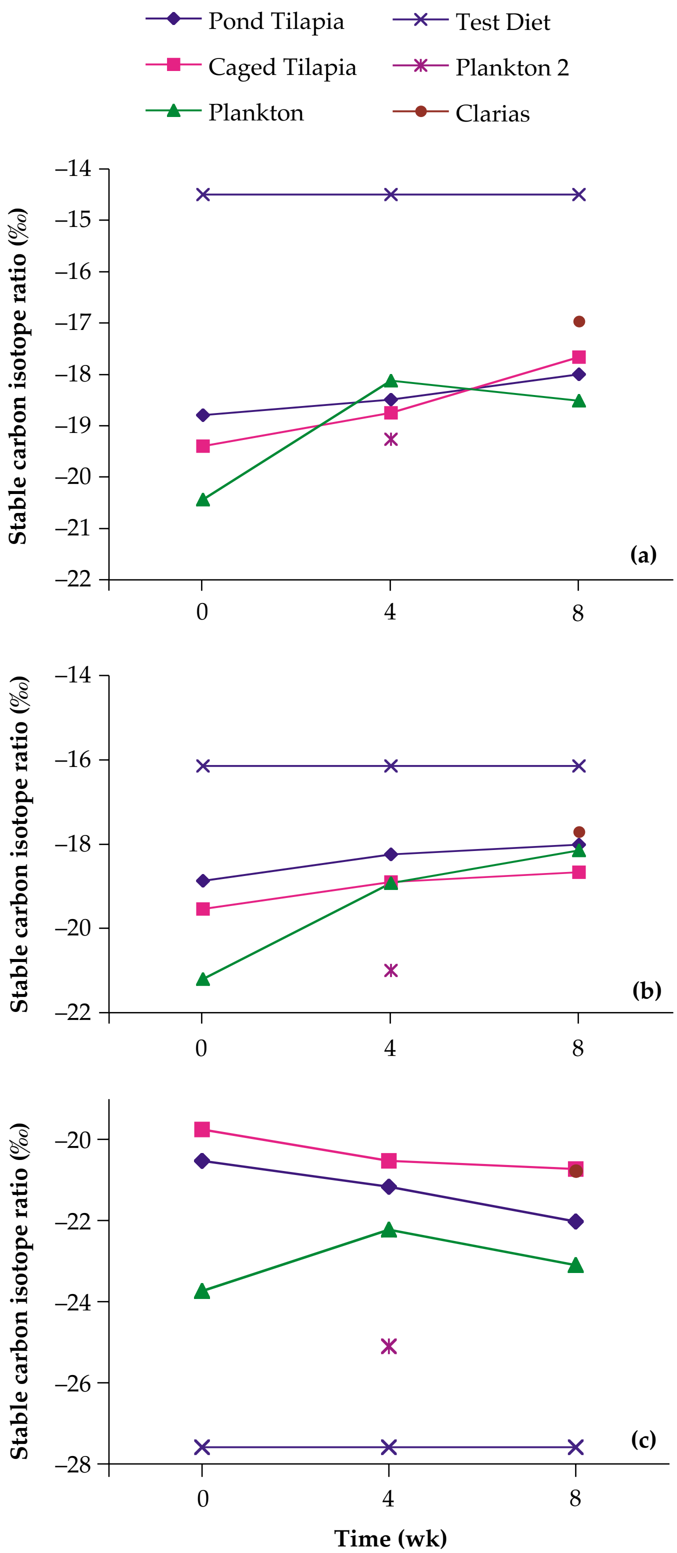

Figure 1. Mean $\delta^{13}C$ values over time in ponds that received (a) the test diet, (b) pig finisher, and (c) rice bran.

Figure 2 shows the change in $\delta^{15}N$ values in ponds receiving each of the diets over time. The isotope values for the test diet and plankton overlapped and were both lower than the values for free-swimming and caged tilapia (Figure 2a). The $\delta^{15}N$ value of the free-swimming tilapia fluctuated more than that of the caged tilapia, indicating a more varied diet. The free-swimming tilapia appeared to be tracking the plankton and/or diet in the middle of the study, but then the final value became more positive, indicating a greater emphasis on the prepared diet at the end of the study, or possibly the assimilation of other more isotopically positive nutrient sources.

The $\delta^{15}N$ of the free-swimming and caged tilapia in ponds that received pig finisher were very similar throughout the study (Figure 2b). They became slightly lower by the end of the study. The diet and plankton values were not distinct at any point in the study, although both were much lower than those of the fish. It was not possible to determine the relative contributions of the plankton and pig finisher to the nutrition of the tilapia using $\delta^{15}N$ in this case. The $\delta^{15}N$ of the plankton and rice bran were distinct in ponds that received rice bran (Figure 2c). However, the $\delta^{15}N$ of the free-swimming and caged tilapia changed less in this treatment over time than in any of the others. The $\delta^{15}N$ of the plankton declined markedly during the study, but this decline was not reflected in the $\delta^{15}N$ values of the fish (free-swimming or caged). Again, the lower growth rate of fish fed rice bran compared to those fed the test diet or pig finisher limited the ability to discriminate between the food sources of the fish using stable isotopes.

The isotope technique is more effective in pinpointing nutritional inputs of an animal when the inputs are isotopically distinct from each other and from the animal itself (Anderson et al., 1987). The discriminating power of the isotope technique may be improved in future studies by fractionating the plankton into different categories consistently before isotope analysis, as fish gut content analysis in the Eighth Work Plan indicated that there were major differences in the relative use of zooplankton and phytoplankton for *Oreochromis* and *Clarias* in the different treatments (Lochmann and Perschbacher, 2000). The nitrogen isotope data clearly provide a distinct data set from the carbon isotope data. The point-in-time data for *Clarias* indicated that the dietary habits of all of the fishes had considerable overlap. However, in this study there were not sufficient data collected over time on *Clarias* to elucidate dietary habits in relation to trophic level of the fishes that were fed different diets.

Anticipated Benefits

Production efficiency of *Oreochromis* and *Clarias* can be improved once the quantitative importance of different nutrients under defined experimental conditions is established

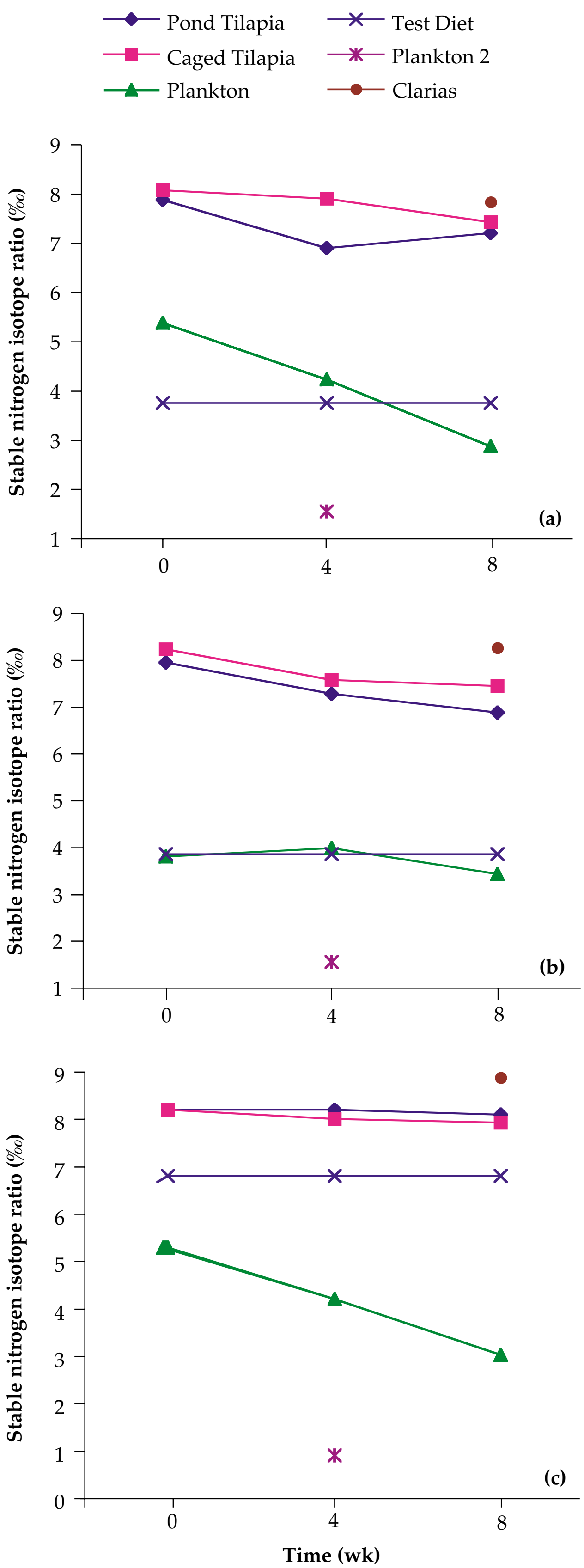

Figure 2. Mean $\delta^{15}N$ values over time in ponds that received (a) the test diet, (b) pig finisher, and (c) rice bran.

using the isotope technique in conjunction with comprehensive production data. Furthermore, the procedures used to define the importance of various components in this aquaculture production system may be modified and applied to other systems in other regions.

LITERATURE CITED

Anderson, R.K., P.L. Parker, and A. Lawrence, 1987. A $^{13}C/^{12}C$ tracer study of the utilization of presented feed by a commercially important shrimp *Penaeus vannamei* in a pond growout system. J. World Aquacult. Soc., 18(3):148–155.

Association of Official Analytical Chemists, 1984. Official Methods of Analysis, Fourteenth Edition. Arlington, Virginia, 1,141 pp.

Folch, J., M. Lees, and G.H. Sloane-Stanley, 1957. A simple method for the isolation and purification of total lipids from animal tissue. J. Biol. Chem., 226:497–509.

Lochmann, R. and P. Perschbacher, 2000. Nutritional contribution of natural and supplemental foods for Nile tilapia: Stable carbon isotope analysis. In: K. McElwee, D. Burke, M. Niles, X. Cummings, and H. Egna (Editors), Seventeenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, pp. 29–31.

Lochmann, R. and H. Phillips, 1996. Stable isotopic evaluation of the relative assimilation of natural and artificial foods by golden shiners (*Notemigonus crysoleucas*) in ponds. J. World Aquacult. Soc., 27(2):168–177.

Lochmann, R., H. Phillips, S. Dasgupta, D. Gatlin, and S. Rawles, 2001. Stable carbon isotope ratios and standard production data as indices of golden shiner, *Notemigonus crysoleucas*, performance in pond feeding trials. J. Appl. Aquacult., 11:21–34.

Schroeder, G.L., 1983. Stable isotope ratios as naturally occurring tracers in the aquaculture food web. Aquaculture, 30:203–210.

Yoshioka, T., H. Hayashi, and E. Wada, 1989. Seasonal variation of carbon and nitrogen isotope ratios of plankton and sinking particles in Lake Kizaki. Jap. J. Limnol., 50:313–320.

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